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Review

Chromosome dynamics and folding in eukaryotes: Insights from live cell microscopy

Kerstin Bystricky

[a] University of Toulouse, UPS, Toulouse, France
[b] Laboratoire de Biologie Moléculaire Eucaryote, CNRS, LBME, 31062 Toulouse, France

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ABSTRACT

How chromosomes are folded and how this folding relates to function remain fundamental questions. Answering them is rendered difficult by the stochasticity of chromatin fiber motion which inevitably results in heterogeneity of the populations analyzed. Even if single cell analyses are beginning to yield precious insights, how can we determine whether a snapshot of position is related to function of the probed locus or cell-type? Fluorescence labeling of DNA at single or multiple loci allows determination of their position relative to nuclear landmarks and to each other, enabling us to derive physical parameters of the underlying chromatin fiber. Here I review the contribution of quantitative spatial and temporal analysis of labeled DNA to our understanding of chromosome conformation in different cell types, highlighting live cell imaging techniques and large scale geometrical analysis of multiple loci in 3D.

1. Introduction

Chromosomes take up characteristic positions within the eukaryotic nucleus [1–3], yet the chromatin fiber is in constant motion and its structure is sensitive to biological processes. The spatial organization of the genome can be studied on several scales: composition and density of nucleosome particles, looping of fibers, formation of chromosome domains and positioning relative to landmarks of nuclear architecture. These properties are related both to each other and to chromatin function, making it important to keep a clear distinction between them (Fig. 1).

In the past two decades, fluorescence microscopy approaches to studying chromatin have benefitted from significant improvements in hardware and in computational image analysis, as well as from new tools for labeling DNA in vivo. Fluorescence imaging both of fixed cells, using in situ hybridization and immunofluorescence, and of living cells, using tracking of loci labeled with protein chimaeras, have revealed preferential positioning of specific loci and distribution relative to each other. It has also brought to light correlations between chromatin organization and local or global changes in DNA metabolism such as transcription and repair [4–10].

This review focusses on the contribution of fluorescence imaging to in situ analysis of DNA dynamics and chromosome folding in yeast and in mammalian cells, the most intensively studied models, while acknowledging that studies of worm, drosophila and plant cells have also provided important insights [see for example [11–13]].

Three major aspects will be discussed:

- Intrinsic properties of the chromatin fiber, compaction and flexibility.
- Chromosome conformation and folding.
- Chromatin behavior during transcription activation.

2. Main text

2.1. Visualizing DNA in living eukaryotic cells

Nuclear DNA can be visualized in bulk either directly by incorporating injected or transfected fluorescent nucleotides during replication, or indirectly by expressing fluorescent histones. Visualization of DNA at specific genomic loci requires labeling techniques that create a fluorescent focus detectable above background levels. Several systems useful for live cell imaging have been developed: FROS (fluorescent repressor operator system) [14], which is based on the insertion of numerous bacterial lac, tet or lambda operator sequences to which fluorescent repressor fusion proteins bind; CRiSPR/inactive Cas9 [15,16] or TALE [17,18], which when fused to fluorescent proteins enable visualization of the naturally occurring repetitive sequences they target;

E-mail address: kerstin@biotoul.fr

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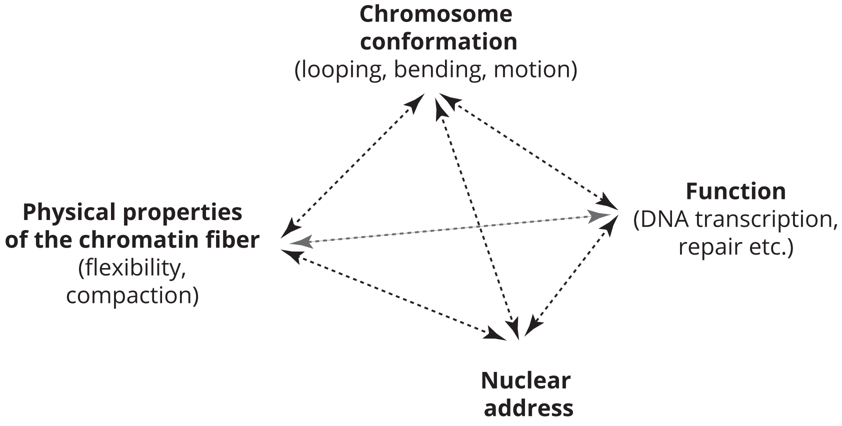

Fig. 1. Cause or consequence? Interdependence of the main parameters governing dynamics of the nucleosome fiber.

suntag [19] which is based on the binding of multiple fluorescent antibodies to a protein bound to a DNA locus; and ANCHOR (ParB/INT), based on amplifying the signal from a small binding site of less than 1 kb (INT) by oligomerization (spreading) of the specific binding protein (ParB) [20]. All these new DNA based tools are derived from the microbial world, a deep reservoir of natural innovation whose potential is no doubt far from exhausted.

Ideally, a labeling system should disrupt neither locus nor neighboring DNA, persist for the duration of the experiment and require no or minimal modification of the genome (Table 1). FROS has proven to be a valuable tool for determining position and dynamics of DNA loci in yeast and to a lesser extent in drosophila and mammalian cells. Three systems of distinct specificity have been developed for use alone or in combination, and a variety of fluorescent proteins of separate emission spectra is available for visualization of each of the three repressor proteins [14,21–23]. In yeast for example, analysis of the motion of FROS-labeled sites led to identification of the pathways involved in positioning telomeres near the nuclear periphery, and to charting changes in mobility associated with replication, with homologous recombination -mediated repair of double-strand breaks, and with regulation of transcription upon change of carbon source [7,14,20,24–27]. The motion of DNA in ectopically inserted operator arrays was shown to be constrained near nucleoli and the nuclear envelope of mammalian cells [28]. Plasticity of chromatin condensation was seen to be correlated with differentiation of ES cells [29], and the dynamics of X chromosome pairing was revealed [30]. In addition, the consequences of DNA cleavage and induced translocations were assessed in living cells [31,32]. Despite these successful applications, use of FROS in higher eukaryotic cells has often proven difficult, owing to the highly repetitive nature of the operator arrays. In particular, the sheer size of the inserted sequences and the tight binding of the repressor proteins can interfere with normal chromatin structure and function, creating fragile sites and perturbing transcription regulation [33,34].

The inactive Cas9 and TALE fluorescent fusion systems avoid this drawback by targeting natural sequences, thus circumventing the need for insertional disruption of the genome. Their use to label non-repetitive sites is however hampered by the need to amplify the signal to visible levels by multimerizing the constructs. Amplification through multiplexing Cas9 involves generating numerous adjacent sites of local DNA unwinding for guide RNA annealing over several kb of sequence, potentially creating DNA damage and nucleosome displacement. Consequently, use of these systems has so far been restricted largely to labeling of naturally repeated sequences e.g. telomeres [35]. Suntag is a non-invasive technique but appears thus far to be reserved for selected proteins that retain their function within the construct [19]. The INT sequence of the ANCHOR system has the advantage of being short, non-repetitive and non-disruptive of chromatin structure [20]. In

Table 1
DNA labeling tools for use in eukaryotic living cells.

System	Origin	Applications, advantages, drawbacks	Genome editing	References
FROS (fluorescent repressor operator system)	*E. coli* chromosome (lactose); Tn10 transposon (tetracycline gene); phage lambda	Yeast, difficult in metazoans; replication blockage (fragile sites); potential chromatin disruption, interference with transcription	Required; insertions 5-10 kb of arrays of repetitive DNA sequences	[14,23,22]
TALE (transcription activator like effector proteins)	*Xanthomonas oryzae* transcription activator	Any cell type; restricted to repetitive sequences	No	[17,18]
CRISPR/Cas9	Streptococcus pyrogenes (Sp), *Neisseria meningitidis* (Nm), and *Streptococcus thermophilus* (St1)	Any cell type; restricted to repetitive sequences or numerous sites (several kb); local DNA unwinding/triple helix formation	No	[15,16]
Suntag	Antibody	Potentially any cell type; very specific, lack of versatility; final complex very large (up to 1400kDa); high probability of instability, aggregation and interference with function of protein studied	Required; insertion of protein binding sites	[19]
ANCHOR (ParB/INT)	Burkholderiaecae chromosome partition systems	Any cell type; versatile; negligible interference with DNA processing, transcription	Required; insertions of 0,4-1 kb unique sequences	[20]
Fluorescent dNTPs		Bulk labeling; whole genome; unspecific; labeling requires single cell injections or 'rubbing'	No	[103]
Histones (H2B-GFP etc.)		Bulk labeling; whole genome; unspecific; stable fluorescence; photoactivatable fusion allows activating individual chromosomes	No	[49,93]

principle it can be used to label any locus. Owing to the variety and specificity of the existing ANCHOR systems and of those still being developed, simultaneous labeling of as many loci as there are distinguishable emission spectra seems within reach. Finally, the development of new fluorescent dyes [36] has the potential to expand application of each of these visualization techniques.

2.2. Chromatin compaction and flexibility parameters based on distance measurements and polymer modeling

The exact nature of the folding of the chromatin fiber is a matter of current debate (Fig. 2A). Several higher order structures are able to form in vitro [37–40] but ultrastructural analysis of nuclear chromatin cannot, at least in most cell types, identify fibers beyond the nucleosome level [41,42]. Electron spectroscopic imaging, which distinguishes phosphorus atoms and provides a trace of the path of the DNA in a chromatin fiber [41], and cryoelectron microscopy of frozen nuclei [43] have also indicated that chromatin in cultured mouse embryonic fibroblasts and naive mouse tissues may be organized almost exclusively as 10 nm fibers. These and other results have sparked lively discussion as to the existence of a hierarchical organization of higher order chromatin structures in both interphase and mitotic chromosomes [43]. Application of biochemical, biophysical, and molecular biological techniques as well as imaging have led to the proposal of numerous models of chromosome fiber folding: molecular crowding, fractal, strings-and-binders and random polymer, to name a few (reviewed in [44,45]; and see below). Microscopy approaches would appear to have the greatest potential for determining the validity of these models.

Determining the physical distance between two fluorescently labeled genomic loci at varying genomic distances provides a measure of chromosome compaction [46–48]. The relationship between the genomic and physical distance indicated that interphase chromatin behaves essentially as a random polymer up to 1–2 Mb [46,49,50]. For genomic distances greater than ~1 Mb, description of the organization of chromatin requires inclusion of additional parameters. The simplest model that takes long-range interactions and loop formation into account is a random walk. To combine structural flexibility with a high degree of compartmentalization characteristic of nuclear organization, the multi-loop-subcompartment model was proposed. This model predicts formation of a rosette-like structure of chromatin comprising numerous 120 kb-loops [51]. Along the same lines, the random loop model describes key properties of the chromatin fiber at scales ranging from 0.5 Mb to 75 Mb, suggesting the existence of 10–30 loops per 100 Mb [52]. At shorter scales (0.5–3 Mb), this model also posits sub-chromosomal domains in order to account for differences in chromatin compaction. It becomes apparent that the parameters of intrinsic compaction and flexibility of the chromatin fiber at short scales, and the organization of these fibers at the megabase level should be analyzed separately, always bearing in mind that the higher order organization is not independent of the folding properties of the fiber (Fig. 1).

At sub-Mb scales, in fixed or living cells of the yeast *Saccharomyces cerevisiae*, measurements of interlocus distances in 3D as a function of genomic distance between fluorescently labeled loci (14–500 kb) have served to model chromatin as a series of linked but independent segments (the steps of a random walk), i.e. as a worm-like chain [50]. Two main parameters describe the properties of the chromatin fiber: the compaction ratio, which gives the number of nucleosomes per unit length of DNA, and the persistence length (Lp), which is a measure of the flexibility of the fiber. Below the Lp, a polymer ceases to behave elastically and can be treated as a stiff rod; above the Lp the capacity to bend and form loops can be considered as unconstrained. The first modeling of chromatin based on distance measurements from FISH and FROS data in yeast evaluated chromatin as a rigid polymer with Lp ~ 200 nm and a nucleosome density of 7–10 per 10 nm [48]. However, subsequent polymer modeling of contact frequencies between loci along yeast chromosome III (320 kb) obtained using 3C [47] suggested an Lp closer to 100 nm and a packing ratio of 1–3 nucleosomes/10 nm. Both sets of compaction and Lp values are routinely used to derive models from chromosome conformation capture ('C') techniques (for example see [53–55]) and to simulate genome-wide chromosome organization [56], despite the fact that yeast chromatin may be even more flexible (see below, [57]).

Polymer modeling usually relies on the mean or median value of measured distances. However, intrinsic motion of chromatin

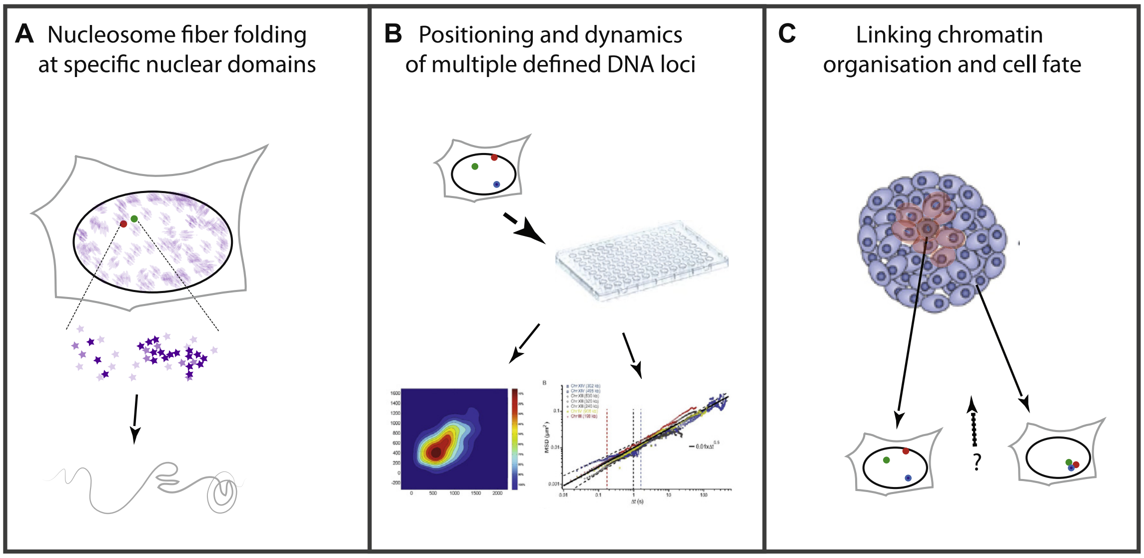

Fig. 2. Live cell imaging approaches for determining physical properties of the chromatin fiber at defined loci (A) to determine statistically significant relative positions, (B) to infer chromosome conformation using high throughput technologies for determining density maps of relative positions of multiple loci or real time imaging of fluorescently tagged loci (examples of representative results from studies in yeast are shown [57,22]), and (C) to track changes in position and motion during cellular differentiation, reprogramming or development in multicellular systems, organs or whole animals.

results in significant cell to cell heterogeneity and thus a broad distribution of distances measured between two fluorescent DNA loci (Fig. 3A and B). Distance distributions have not so far been systematically taken into consideration in determining parameters of chromatin compaction and folding.

Visual models (cartoons or 3D renderings obtained from computation) represent an average conformation based on the most frequently observed events. Such average conformations are unlikely to exist, other than fleetingly at very low frequency within the nucleus. Modeling single cell Hi-C data provided a striking demonstration that the same chromosome, mouse chromosome X, can adopt very different conformations in each of the cells analyzed [55]. Giorgetti et al. [58] tackled this problem from a different angle. In a population-based 5C study, they developed a thermodynamic model based on Monte Carlo sampling of fiber states to describe folding of a 260 kb domain encompassing the Tsix locus in mouse ES cells. They were able to not only correlate distances derived from modeling 5C data with mean 3D distances from seven high-resolution FISH probes (genomic separation of 19–250 kb), but also to reproduce measured FISH distance

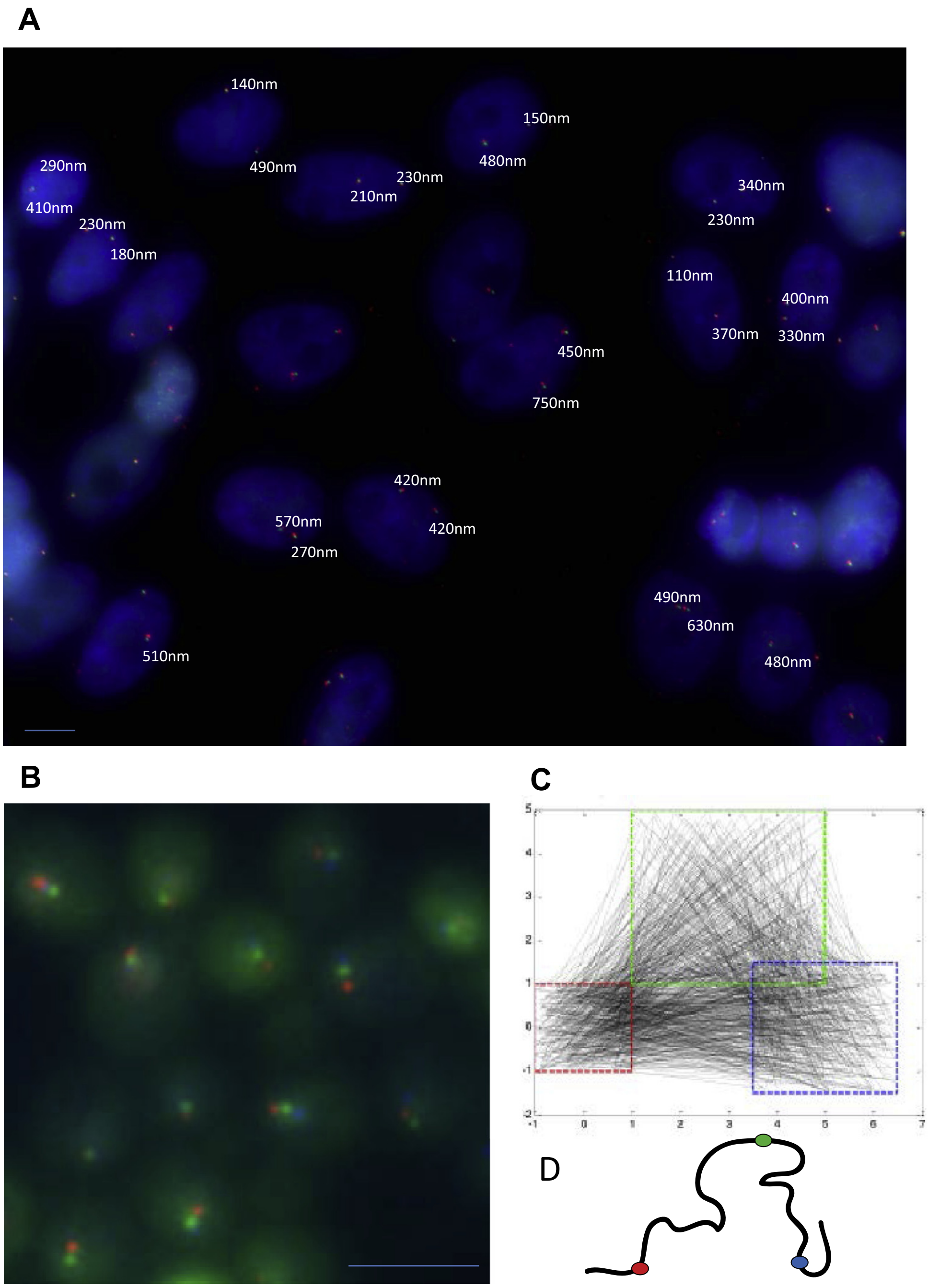

Fig. 3. Stochasticity of chromatin in living cells. (A) Representative image of a two color FISH performed on human mammary tumor, MCF7, cells (probes to 3′ and 5′ end of the progesterone receptor gene at 90 kb distance; physical separation from 3D structure preserving FISH was measured using NEMO and plotted for each of the two alleles). Size bar 5 µm. (B) Representative image of a culture of exponentially growing yeast cells bearing three FROS labels on yeast chromosome III at *HML* (red:128xtetO; RFP-TetR), *MAT* (green:64xλO; λCi-YFP) and *HMR* (blue:256 lacO; CFP-lacI). Size bar 2 µm. (C) Relative positions of the three loci (from B), modeled from $n = 500$ positions, iteratively matched to measured data [22]. Each locus can be found at any position within a defined zone (survival zone; arbitrary units). (D) Schematic drawing of the path the chromatin fiber could take between multiple loci constrained to their survival zones.

distributions by computing statistical fluctuations between 5C models. In this study, the chromatin fiber was simulated as a series of 3 kb beads of diameter 53 nm ($\sim$15 nucleosomes), which precludes determination of detailed fiber folding properties. Nevertheless, their model supports the existence of a 30 nm higher order structure, rather than a more relaxed 10 nm fiber, at the Tsix locus in mESC cells. This raises further questions since ES cell chromatin is usually considered as decondensed and lacking higher order conformations.

Knowing the path of the fiber between two loci inside the nucleus would thus significantly improve our ability to interpret imaging and 'C' data. With the advent of super-resolution microscopy, imaging of histones provides information about regional differences in packing and mobility within the imaged nuclei [59–61]. The next challenge is thus to combine bulk labeling with specific tags to zoom in on a defined DNA locus within a chromatin domain (Fig. 2A).

2.3. Flexibility and confinement defined by fiber motion

Most of the spatio-temporal fluctuations of chromatin result from its diffusive behavior, but there have been few attempts to exploit those fluctuations to calculate the intrinsic physical parameters of chromatin (Fig. 2B). The motion of FROS-labeled genomic sites was shown to undergo normal Brownian motion at small time scales and to be confined in volumes of $R = 0.3\ \mu m$ [24,55]. Single-particle tracking of nucleosome motion revealed local dynamics of these particles in interphase [60] and is consistent with the random motion of FROS labeled chromosome segments. The most recent live cell distance measurements were based on tracking of 20 loci distributed among yeast chromosomes III, IV, VI, XII, and XIV in live yeast [62,63] over an extended temporal range of more than four orders of magnitude. These measurements demonstrated that the behavior of yeast chromatin conforms to the Rouse model of polymer motion [57]. The Rouse model assumes that the motion of chromosome loci is dominated by elastic interactions between nearest-neighbor chromatin segments. Applying this model to the mean square displacement of the tracked genomic loci yielded an $Lp \ll 30$ nm and nucleosome density 1-3 per 10 nm, fully consistent with a 10 nm fiber and compatible with observations from electron-microscopy. Re-analysis of the original FISH genomic vs physical distance data set from [48] using the Rouse model gave a good fit. The apparent discrepancy in compaction and Lp values from the same distance data set can thus be explained by the application of different polymer models which all inevitably include certain assumptions (bead size and unit length, volume exclusion, parameters describing bead interactions etc.), and illustrates the need to analyze chromatin motion as well as inter-locus distances [48,57].

In contrast to relatively free moving chromosomal sites, dynamics of spatially confined polymers, such as telomeres which are frequently near the nuclear periphery in yeast, or rDNA proximal loci which are next to the nucleolus, generally exhibit reduced dynamics [27,63,64]. Constrained motion could reflect hindrance imposed by physical attachment, a modification in the compaction parameters of the fiber or a combination of both. FROS-labeled loci in mammalian cells were also reported to be less dynamic [65] when next to the nuclear envelope, whereas Cas9-GFP or TALE-GFP marked telomeres tended to be rather mobile within the nuclear lumen in human cells [15]. Motion of randomly labeled sites [65] or of fluorescent ANCHOR tags next to specific genes (my group's unpublished data) is heterogeneous within a cell population, in which their localization with respect to nuclear compartments is likely to vary. Nuclear location clearly influences conformation of eukaryotic chromosomes. However, the repercussion on their freedom of movement seems to be mainly a consequence of confinement rather than a change in physical parameters of the fiber itself.

2.4. Geometrical analysis using multiple labeled loci to probe chromosome folding

The inter-locus distances usually examined fall between a few kb and several Mb. These distances are much greater than the Lp, the principal parameter used to characterize flexibility and to describe the path taken by the fiber between the two labeled sites. Extreme variability of fiber conformations is easily illustrated by the range of 3D distances measured between two FISH probes hybridizing to a 90 kb segment in human mammary tumor cells (Fig. 3A). Distances differ even between the two alleles within the same nucleus. The inclusion of a third point opens the way to more precise estimation on fiber folding and flexibility. Yet, so far only a few studies have used three distinct loci to analyze chromatin conformation. In fixed, mammalian cells, distances and angles within a triangle formed by three DNA probes were measured to determine changes in chromatin domain condensation [66]. In living mammalian cells, three DNA regions were detected simultaneously by employing inactive Cas9 fluorescent fusion proteins, but although indicated, changes in chromatin compaction were not analyzed quantitatively [16]. In yeast, my group has used three FROS DNA labeling systems simultaneously to do just that (Table 1; [22]). Different sets of three loci separated by 80–100 kb on chromosome III were tagged. The relative positions of the three tags varied greatly from nucleus to nucleus as illustrated here by a single image of an exponentially growing yeast culture (Fig. 3B). Triangulation of the positions of the three DNA loci enabled determination of angles and distances between them. Clustering of these interdependent data finally let to the identification of differential, transient folding of the labeled chromosome [22]. We further developed a mathematical polymer model to determine the zones (survival zones; Fig. 3C) that the three linked loci can occupy relative to each other. A theoretical path of the chromosome fiber between the three survival zones (Fig. 3D) supports the view that yeast chromatin is flexible and only moderately constrained. This approach should allow physical parameters of the chromatin fiber to be defined in vivo and in any cell type (Fig. 2B).

Furthermore, a triangulation procedure enabled cell type-specific differences to emerge [22]. This was unexpected, because previous studies using one or two labels were either unable to demonstrate such a difference or reported minor differences that could not be attributed to specific conformations [25,67,68]. Chromosome III, attached to the spindle pole body by microtubules bound to its centromere, occupies a limited space within the yeast nucleus. Thus, the average positions of chromosome III will be similar most of the time, independently of cell type or physiology. Yet a few of the snapshots of the positions of the three labeled mating type loci were characteristic of one or the other cell type [22]. To infer chromosome conformations from those snapshots, we used the relative distances and angles formed by three points in space to identify chromosome conformations which are possible but not frequently detectable in a dynamic system. These conformations are transient in comparison to average conformations which rely on stable positions. During the gene conversion event or mating type switch, one of the silent mating type loci contacts the broken *MAT* locus upon induction of a double strand break. This transient event may be dominated by a particular conformation of chromosome III. Therefore, even if we cannot capture events directly, we want to find out whether they are possible by analyzing the events we can detect.

The recent elaboration of systems that expand the number of loci that can be labeled simultaneously (Cas9, ANCHOR, etc; Table 1) should allow many further analyses of this type, and so extend our ability to relate chromatin conformation to function. Their analysis requires imaging large numbers of single cells and further developing computational tools to analyze, interpret and statistically compare the results (Fig. 2B).

2.5. Clustering of single-cell images to identify infrequent conformations

Much of our recent knowledge of chromosome folding is derived from data obtained at the genomic level using methods that rely on protein-DNA cross-linking [see reviews in this special issue] [54,69–71]. 'C' techniques have provided a wealth of information on three-dimensional long range molecular contacts between genomic DNA regions, and methods based on chromatin immuno-precipitation have revealed the genomic distribution of epigenetic modifications and their protein machineries. Interaction maps contain a great deal of structural information but their interpretation is hampered by the need to average different structures within a population. In addition to computational modeling, this population-based structural data must be complemented with imaging approaches to obtain true physical distances between specific loci. Although contact frequencies have been shown to reflect physical proximity [26,58], correlating them with preferential positions in the nucleus from cell populations has its limitations [72]. Transforming interaction frequencies into spatial distance has been achieved by various computational approaches [73–76], but remains a matter of debate and prone to interpretation of experimental artefacts, such as the importance of formaldehyde crosslinking or the resolution of multi-color imaging. Numerous structures of the genome have been generated from randomly permutated datasets at different resolutions and algorithms, now providing positional information at low computational cost [75,77]. Obviously, a virtually unlimited number of models can be obtained from such computational recording of experimental data [53,54,56,73,78,77,74,75,79]. It will hence be necessary to cluster datasets and classify resulting models to determine which ones are likely to be correlated with function. For example, models of the Tsix gene locus in mouse ES cells based on 5C data revealed that its folding can be compressed or elongated depending on transcriptional status [58]. Similarly, subsets of 5C-derived models of yeast chromosome III reveal mating type specific differences in the conformation of the chromosome and a structural role for a small sequence element, the recombination enhancer (Belton et al., unpublished). The identification of structural features that are functionally relevant, but not necessarily the most frequently observed ones, relies on analysis of single cells [55,80]. However, the probability that the snapshot of a single cell at the moment of fixation or imaging is representative of a meaningful event can be very low, because many events are very short lived. This is illustrated by the small percentage (~5%) of imaged nuclei in which Fanucci et al. detected colocalization of three mRNA signals from co-regulated genes using in situ hybridisation [81]. In order to detect rare events and, more importantly, enable statistically significant comparison of data in different conditions and cell types, high throughput imaging techniques with sufficient resolution to detect point like signals (spots) in 3D (Fig. 2B) need to be developed. Although high-throughput sequencing using microtiter plates and microfluidic devices has advanced apace, few high through-put imaging approaches for detection of DNA or RNA within nuclei have so far been reported. So it is encouraging that in two recent pioneering studies the frequency of chromosomal translocations between FROS labeled sites and of RNA FISH signals could be assessed [31,82]. This work, though not directly focused on transcription control, provides a technological framework for studying relationships between DNA function and chromatin dynamics.

2.6. Tracking DNA dynamics during transcription in living cells

A central issue in chromatin dynamics is its relationship to gene expression. Large scale changes in the average position of genes relative to chromosome territories or the nuclear periphery in response to transcription activation may relate to local concentrations of transcriptionally active enzymes, in particular of polymerase II [83]. On a local scale, transcription activation requires chromatin remodeling and decondensation. Taken together, these observations support the view that transcription activation is accompanied by an increase in local mobility. This has been tested experimentally by tracking DNA constructs composed of arrays of an inducible gene and FROS repeats. These were seen to migrate over several hundred nm in human cells within a few hours [84–86]. A more direct method for linking chromatin dynamics is the visualization of nascent mRNA using the MS2 and PP7 systems. Fluorescent derivatives of the MCP (MS2 coat protein) and the PCP bacteriophage proteins expressed as fluorescent fusion proteins bind stably to repeated specific hairpin structures on mRNA. Production and mobility of RNA newly synthesized from single genes can be measured with high precision [87–89]. RNA production oscillates between "on" and "off" states, which may be linked to conformational changes related to chromatin remodeling [90]. Turning to the role of histone modifications, Kimura's group [91,92] developed a method based on fluorescently-labeled antigen binding fragments (Fabs), which permits monitoring of the distribution and general level of endogenous histone modifications on an array of ~200 MMTV reporter constructs in living cells. Since Fabs bind their target transiently, the ratio of free to bound fragments varies with changes in gene transcription. When used in conjunction with newly developed DNA tagging systems (Table 1), Fab-based live endogenous labeling should prove extremely valuable for elucidating the role of histone modifications in gene expression in living cells.

Local fluctuation of individual nucleosomes (50 nm movements/30 ms) in both interphase and mitotic chromatin is caused by confined Brownian motion [49]. This local nucleosome movement can assist the dynamics of transcriptional complexes and their targeting to specific DNA sites. Monitoring these processes will be facilitated by new technical developments. Super resolution imaging methods such as STORM (Stochastic Optical Reconstruction Microscopy) and PALM (PhotoActivated Localization Microscopy) provide unprecedented insights into how chromatin is folded and organized. For example, Recamier et al. have computed the 3D distribution (Ripley distribution K(r)) of distances between two points of photoactivatable H2B-GFP [93]. They observed dynamic evolution of chromatin sub-domain compaction, leading to a model in which chromatin organization is actively maintained by enzymes acting upon chromatin compaction. Indeed, in yeast, addressing the chromatin remodeling complex INO80 via LexA binding sites near a reporter gene enhanced local chromatin dynamics of the lacO FROS near the targeted gene (the diffusion coefficient D and the radius of confinement R_C increased by ~20%). However, enhanced dynamics of the target site did not directly parallel polymerase elongation activity at the reporter gene [94]. It is important to note that the lexA-INO80 fusion was continuously expressed and present at the tracked PHO5 locus activated in these cells grown in low phosphate medium. It would hence be informative to determine the variations in the motion of the tracked FROS labeled locus directly, in real time, during phosphate depletion. In mammalian cells also, chromatin decondensation was sufficient to alter average positions of some reporter genes independently of their transcriptional activity [4]. In both cellular systems, it remains to be determined whether a change in mobility and local decondensation result from or cause alterations in the actual physical parameters of the chromatin fiber. Nonetheless, increased mobility or large scale relocalization seem to precede transcription per se. Observing consequences of transcription initiation and elongation on chromatin motion and properties will rely on imaging of DNA loci labeled close to the transcription start site in an inducible system and within the first minutes of activation, the time during which co-factors bind and remodeling occurs (for example in hormone induced cells; [95,96]). Over long time periods, during differentiation, reprogramming and developmental processes, analyzing potential changes in position and motion of one or multiple DNA loci undergoing transcriptional changes, will rely on DNA tags stably inserted and as inert as possible with respect to the tracked locus's function (Fig. 2B and C). Optimizing spatio-temporal resolution in living single cells or multicellular samples will be necessary.

3. Conclusions and perspectives

We are currently at the stage where it is difficult to derive reliable correlations between dynamics and function of a specific DNA locus; establishing causal relationships is a challenge we have yet to meet. Nevertheless, high resolution and high throughput live cell imaging could bring it within grasp. To advance our understanding of genome organization, we need tools for defining subpopulations of nuclei with locus distributions characteristic of particular processes; essentially, we need the means of determining spatial folding features of chromosomes that go beyond contact frequencies and pair-wise distance measurements.

Active and inactive segments of the genome appear spatially separated while co-regulated sites sometimes, but not necessarily, group within the same topological domains, which may contribute to their coordinated expression or silencing [83,6,97]. It is less clear whether changes in chromosome conformation can drive nuclear processes and, by doing so, regulate them (Fig. 1); [98]). If so, which sequence of events could link function and kinetics at short time scales? Motion associated with transcription may depend on fiber compaction and flexibility. These avenues are far from being fully explored.

New tools have to be united and used for the study of gene regulation in the context of normal and pathological tissues. Improvement of imaging techniques, the design and synthesis of new fluorescent proteins and dyes with greater emission yield and wavelengths ranging from UV to infrared on the one hand and, on the other, genome editing (TALE, CRISPR) and labeling systems now offer unlimited opportunities to probe chromatin position and dynamics in space and time.

Many data tend to support a rather homogenous, highly flexible chromatin fiber at the nucleosome level modeled as homopolymers. Would heteropolymer modeling give extra insight on higher order folding or is there no compact chromatin fiber state? Dynamics and folding capability of the chromatin fiber are influenced by spatial confinement and higher order structural features such as loops and attachment sites. Nuclear compartments and protein aggregates likely affect chromosome conformation, but are difficult to include in present models as they remain invisible to 'C' techniques. Live cell imaging of tagged DNA loci and nuclear compartments is thus essential to determination of the impact of potential interactions between these compartments and chromosomes. These interactions are likely to evolve during cellular differentiation as illustrated by inversion of heterochromatin positions within nuclei of rod photoreceptor cells in nocturnal animals [99], or formation of SAHF (Senescence Associated Heterchromatin Foci). SAHF are hallmarks of senescent cells that result from a reorganization of heterochromatin [100] and altered histone modifications which seem to play a role in sequestering proliferation-promoting genes [101]. The identity of the sequences driving this reorganization is not currently known. In yeast, a small sequence element (Recombination Enhancer) was able to alter folding of a chromosome arm [22]. This element is bound by proteins which mediate long range interactions and attachments. In mammalian cells, also, cleavage of a chromatin loop perturbed inter-chromosomal contacts which are likely also to be mediated by proteins, in this case, transcription co-activators [102]. These few examples demonstrate that the conformation of chromosome domains is strongly influenced by external stimuli and dependent on the catalytic function of proteins.

How to probe the role of structure? We need to develop approaches acting upon specific sites which are labeled for rapid in vivo imaging and suitable for high through put analysis. Thus future work (Fig. 2A–C) will require

⇒ Zooming in on the nucleosome fiber at labeled loci to trace the path of the chromatin fiber at the nm level, combining live cell multi-color imaging and super-resolution microscopy.
⇒ Looking at many single cells using high throughput approaches.
⇒ Analyzing single and multicellular systems over time using stable tags and readouts.
⇒ Imagine approaches to probe the effect of structure on function.

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